ANNUAL PROGRESS REPORT (Termination)

Report Prepared by: Jay S. Roth Date: February 1, 1954
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CONTRACT:

ANNUAL RATE: $5,000.00

CONTRACTOR: Division of Biological Chemistry
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TITLE OF PROJECT: The Relation of Methionine to Brain Metabolism.
Objectives: (Studies with Methionine Sulfoximine)

ABSTRACT (OR SUMMARY) OF RESULTS

a. Since start of project: Will be given shortly in final report.

b. During current report period: The metabolism of methionine sulfoximine has been studied by injecting S^{35} labeled compound and determining the partition of S^{35} in the urine sulfur fractions.

PLANS FOR FUTURE:

Immediate: To complete unfinished work and prepare final report.

Long Range:

REPORTS AND PUBLICATIONS:

(During current report period)

The Metabolism of Methionine Sulfoximine. (In preparation for publication.)

The Metabolism of Methionine Sulfoximine

by Jay S. Roth

Introduction

Previous studies on the distribution and excretion of S^{35} labeled L-methionine sulfoximine (MSI) by rats (1,2) indicated that approximately 60 per cent of an injected dose was excreted by 24 hours after the injection. In this report studies have been made of the distribution of the S^{35} in the various sulfur fractions of urine in an effort to determine the extent to which MSI is metabolised by the rat.

Materials and Methods

Male Wister strain rats weighing approximately 200 gm. were injected with 100 mg. S^{35} MSI per Kg. This amount did not produce toxic symptoms although the injected animals did not eat very much during the first 24 hours after the injection. The MSI had a specific activity of 5.72×10^5 counts per min. per mg. and it and all samples were assayed in a gas flow counter with standard geometry. The rats were housed in stainless steel metabolism cages, two to a cage, and urine collected each 24 hours using HCl as a preservative. The animals were fed water and Fox chow meal ad lib. The urine samples were made up to 100 ml.; phosphate was precipitated as $MgNH_4PO_4$ and the sulfur fractionated according to the procedure of Fiske (3) with several modifications. The precipitated benzidine sulfate was filtered on sintered glass filter discs of medium porosity 20 mm. in diameter and 10 mm. high. After drying these could be placed directly in the gas flow counter. When counted the discs were placed in a beaker of boiling water and the benzidine sulfate titrated with 0.02 N NaOH. Where necessary corrections have been made in the counts for coincidence, decay and self-absorption.

In a first experiment 8 rats were utilized and urine collected only during the first 24 hours. The results, shown in Table I, indicated that approximately 5 per cent of the S^{35} excreted during the first day had been oxidized to sulfate. Since the injected MSI consists of two isomers, only one

of which may be biologically active, the material excreted during the first 24 hours after injection would represent mostly the inactive form which is not metabolised. On subsequent days larger percentages of S^{35} might be found in the oxidised form. To test this possibility the procedure was repeated on 6 rats and urine samples collected on the 2,3,4 and 5th day. As the first experiment indicated that large deviations were not to be expected in the individual urines, the samples from each day's collections were pooled and duplicate analyses carried out on each pooled sample. The results are given in Table II. Examination of the data in this table indicated that an increasing percentage of S^{35} was excreted as sulfate from the first to the fifth days of the experiment. By the fifth day, however, the total radioactivity in the urine reached a very low value which seemed to be leveling off. It is possible that the S^{35} excreted as sulfate at this time represented sulfur which had been incorporated into protein and was then being metabolised at a constant rate. The amount of it, however, was not very significant. Of the S^{35} injected, about 92 per cent of the total activity had been recovered by the 6th day. Very little S^{35} was found in the ethereal sulfate fraction.

Chromatographic analysis of Urine

Previous preliminary chromatographic studies (2) had failed to demonstrate large quantities of unchanged MSI in the urine. As the sulfur partition experiments indicated that there was little oxidation to sulfate, the possibility that some altered organic metabolite was present in relatively large quantities, especially in the urine collected the first day, was tested by application of paper chromatography and by use of ion-exchange columns.

An untreated sample of urine, collected during the first 24 hours after injection of MSI was passed through a column of IRA 400, 200 x 8 cm. which had been converted to the hydroxyl form by 4 % NaOH. The material adsorbed by the column was eluted with 0.1 M HCl and the fractions assayed for S^{35} activity. These studies are continuing.

Table I

Excretion of S^{35} During 24 Hours by Rats Injected with S^{35} L-Methionine Sulfoximine

Sample	Total inorganic $SO_4^=$	Activity	Total S as $SO_4^=$	Activity	S^{35} as $SO_4^=$
	mg.	cpm	mg.	cpm	per cent
Rats 1,2	11.1[1]	26,008[2]	14.7	490,730	5.3
Rats 3,4	12.7	25,098	13.9	415,465	6.0
Rats 5,6	11.1	22,286	15.5	468,982	4.8
Rats 7,8	13.3	20,607	16.5	519,153	4.0
					Av. 5.0

1 Twenty ml. sample of urine used for analysis.

2 Corrected for blank. Blank consisted of rat urine to which a similar quantity of S^{35} L MSI had been added.

Table II

Sulfur Partition in the Urine of Rats Injected with S^{35} L-Methionine Sulfoximine

Sample	Inorganic $SO_4^=$	Activity	Total Inorganic $SO_4^=$	Activity	Total S as $SO_4^=$	Activity
	mg.	cpm	mg.	cpm	mg.	cpm
2nd day	4.7[1]	9080	4.8	9160	6.1	109,700
3rd day	5.8	1394	6.0	1475	7.4	11,050
4th day	6.9	590	7.5	660	8.8	3,582
5th day	6.5	434	6.7	464	7.9	1,435

	Ethereal $SO_4^=$	Activity	Organic S as $SO_4^=$	Activity	Per cent of S as $SO_4^=$
	mg.	cpm	mg.	cpm	
2nd day	0.1	80	1.3	100,540	8.4
3rd day	0.2	81	1.4	9,575	13.4
4th day	0.6	70	1.3	2,922	18.4
5th day	0.2	30	1.2	971	32.4

1 5.0 ml. sample; average of duplicate determinations.

Bibliography

1. Roth, J.S., Wase, A., and Reiner, L., Science 115, 256 (1952)

2. Roth, J.S., Eichel, H.J., and Wase, A., J. Biol. Chem. 200, 647 (1953)

3. Hawk, P.B., Oser, B.L., and Summerson, W.H., Practical Physiological Chemistry 12th Ed. p 887 Blakiston (1947)

The Uptake of S^{35} of S^{35}-L-Methionine Sulfoximine by Rat Brain Homogenates

Three rat brains were quickly removed from rats killed by a blow on the head and homogenized with 4 parts of cold Krebs Ringer phosphate solution. One ml. of homogenate was added to Warburg flasks containing 2.0 ml. of isotonic glucose and 4 mg. of labeled MSI per flask. Four flasks were heated to boiling, these served as controls. All of the flasks were then gassed with 95% O_2 and 5% CO_2 for 3 hours at 37° C. with shaking. At the end of this period the contents of the control and experimental flasks (4 each) were pooled and precipitated with an equal volume of ice cold 10% TCA. The precipitates were washed with 3 portions of ice cold 5% TCA and the residues extracted with 95% ethanol and 1:1 boiling ethanol-$CHCl_3$. The protein remaining was dried at 80° C. overnight and then weighed into two Kjeldahl flasks and digested with Pirie s reagent. The sulfate was precipitated as benzidine sulfate using 1.0 ml. of approximately 0.1 M H_2SO_4 as a carrier and collected as previously described on sintered glass plates.

Sample		Counts/min.	mg.S	Counts/mg.
Boiled control	1	503	1.98	254
Boiled control	2	418	2.00	209
Experimental	1	1255	2.42	519
Experimental	2	1453	2.50	582

These results although indicating a significant uptake of S^{35} by brain cannot be taken as conclusive for two reasons. First, it is possible that the increased count in the experimental samples is due to uptake of relatively small amounts of impurities such as methionine sulfoxide which may be present in the sample of sulfoximine. Second, in view of the appreciable count of the boiled controls which may be due to adsorption of the radioactive material by protein it would be necessary to isolate the sulfur containing amino acids from the protein of the experimental samples and prove that the radioactivity was associated with these amino acids. Further studies are planned on the uptake of S^{35} from S^{35} MSI by liver and spinal cord.